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# Insecticide susceptibility in larval populations of the West Nile vector *Culex pipiens* L. (Diptera: Culicidae) in Saudi Arabia

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ABSTRACT

**Objective:** To investigate the susceptibility to some conventional and non-conventional insecticides in laboratory and field larval populations of the West Nile vector *Culex pipiens* L. (*Cx. pipiens*), the dominant species in Jeddah Province, Saudi Arabia.
**Methods:** The tested conventional insecticides were Actikil and Pesgard, while the non-conventional ones were Bacilod, Dudim and Baycidal. Probit analysis and photo-microscopical observations were carried out to shed light on acute toxicity in laboratory and field *Cx. pipiens* strains.
**Results:** *Cx. pipiens* were more susceptible to Pesgard ($LC_{50}$: 0.045 and 0.032 mg/L) than Actikil (0.052 and 0.038 mg/L) and Bacilod (0.129 and 0.104 mg/L), for the field and laboratory strains, respectively. Results showed that treatments with the chitin synthesis inhibitor Dudim and Baycidal evoked morphological effects similar to those induced by other insect growth regulators. According to $IC_{50}$ values obtained (concentration which to inhibit the emergence of 50% of mosquito adults), the compound Dudim (0.000 3 and 0.000 1 mg/L) was more effective against *Cx. pipiens* L. mosquitoes than Baycidal (0.000 4 and 0.000 3 mg/L) for both the field and laboratory strains, respectively.
**Conclusions:** Our results provide baseline data to enhance control programs and orient public health decisions on the selection of pesticides against mosquito vectors in Saudi Arabia.

## 1. Introduction

The development and use of insecticides have produced immeasurable benefits for humankind as they kill unwanted insect pests by disruption of their vital processes through chemical action. Therefore, they have been a major contributor to the upsurge in agricultural productivity over the past three decades. Their use has not only resulted in foodstuffs of the highest quality but also has saved millions of lives through eradication of disease-carrying insects [1].

Mosquitoes are considered as the most important group of insect which have ability to spread the disease in the world [2–4]. Chemical control is one of the main ways to combat mosquito vectors of medical and veterinary importance. In the beginning of the 19th century, the discovery of a group of chlorinated hydrocarbons such as DDT and its derivatives boosted up pest control. Later on, many synthetic chemical insecticides [*i.e.*, organophosphates, carbamates and insect growth regulators (IGRs)] were successfully used to control mosquitoes [5,6]. Over the last century, natural and synthetic insecticides usage

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has increased in the agricultural and public health sectors [7,8]. This has saved millions of tons of agricultural food resources and human lives. However, the introduction of synthetic insecticides has arisen the problem of resistance in vector-borne diseases [9]. Resistance is the developed ability in a strain of insects to tolerate the doses of insecticides, which may be lethal to the majority of individuals in a normal population of the same species. This is reflected in repeated failure of an insecticide to achieve the expected level of control of insects when used according to the product label recommendations and where problems of product storage, application and unusual climatic or environmental conditions can be eliminated as causes of the failure [10]. To overcome these problems, scientists discovered other types of chemicals known as IGRs. IGR is a chemical group of pesticides, which may be used as mosquito larvicides and divided into two families; the juvenoid family, which affects the growth process of the insects, and the chitin synthesis inhibitors, which disrupt the transformation processes. In general, IGRs are safer to fishes, amphibians, mammals and birds, if compared with other pesticides [11]. Pyriproxyfen, belonging to this family, is an IGR that affects the physiology of morphogenesis, reproduction and embryogenesis of insects. It exhibits a high level of activity against mosquito larvae inhibiting adult emergence at low dose [12,13]. Furthermore, Vythilingam *et al.* [14] showed the long-term effectiveness of pyriproxyfen against dengue vectors in Asia. Similarly, Sihunincha *et al.* [15] showed that, pyriproxyfen prevented adult emergence at extremely low concentrations ($LC_{50}$ = 0.012 mg/L) when applied to late mosquito instars. In this research, we determine the susceptibility levels on the West Nile vector *Culex pipiens* L. (*Cx. pipiens*) testing conventional and non-conventional insecticides in Jeddah province of Saudi Arabia. Results provide baseline data to enhance control programs and orient public health decisions on the selection of pesticides in Saudi Arabia.

## 2. Materials and methods

### 2.1. Collection sites

Mosquito larvae were collected from domestic and outside containers around homes throughout Jeddah City, Saudi Arabia, located between latitude 21°29′31″ N and longitude 39°11′24″ E.

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

### 2.2. Mosquito collection and colonization

*Cx. pipiens* larvae were maintained at the laboratory of University of Tabuk under conditions of controlled temperature [(27 ± 1) °C] and relative humidity [(70 ± 5)%] with a constant photoperiod (light: dark = 14 h:10 h). Pupae were transferred from water medium to standard mosquito rearing cages (30 cm × 30 cm × 30 cm). Subsequently, adults were kept in cages and provided with a cotton wick soaked with 10% glucose solution for post-emergence. After a period of 4 days, sugar-fed females were starved for 24 h prior to feed on artificial blood suppliers. Blood-fed females were allowed to assimilate the blood meals for 48 h. Gravid females were given access to oviposition sites consisting of small glass containers (23 cm × 17 cm × 8 cm) lined with filter paper as egg deposition sites. Eggs were dried under laboratory conditions. Samples of eggs from filial generation 13 were hatched in cool sterilized water. Newly enclosed larvae were reared in plastic trays and fed every two days with a powdered mixture of biscuits, dried yeast, and fat-free milk (1:1:1). Late 3rd or early 4th instar larvae of generation 12 were used for larval bioassays. Adult experiments were conducted using sugar-fed (10% glucose solution) 3–5 day-old adults derived from wild larvae after one generation under laboratory conditions.

### 2.3. Insecticides

Conventional insecticides: the Actikil 50% (active ingredient: pirimiphos-methyl 5%), and Pesguard Fg161 (active ingredient: D-tetramethrin 4%; cyphenothrin 6%).

Non-conventional insecticides: Bacilod 5000 ITU (active ingredient: *Bacillus thuringiensis* var. *israelensis*), Dudim 4G (active ingredient: diflubenzuron 4%), and Baycidal (active ingredient: triflumuron 25%).

### 2.4. Larval bioassays

Experiments were conducted following the method by Aziz *et al.* [16]. Batches of 20 larvae were added to glass beakers filled with 200 mL of water containing different concentrations of the five insecticides: *i.e.*, Actikil, Pesguard, Bacilod, Dudim and Baycidal. When larvae were introduced into the beakers, 0.02 g of the powdered mixture was added to avoid death by starvation.

The concentrations applied for conventional insecticides Actikil and Pesguard were 0.02–0.15 and 0.02–0.20. The concentrations applied for non-conventional insecticides, Bacilod, Dudim and Baycidal were 0.05–0.5, 0.000 1–0.00 4 0, and 0.000 2–0.002 0, respectively. These concentrations of each insecticide were tested on fourth instar larvae in five replications, for both laboratory and field-strains. In each case, the same number of glass beakers with the same treatment but without insecticide served as controls. Beakers were inspected 24 h after introduction of larvae and the numbers of dead larvae were recorded.

### 2.5. Data analysis

In the Actikil, Pesguard and Bacilod insecticides bioassay experiment, the numbers of dead larvae were determined after 24 h. Following [17], larvae incapable of reaching the water surface for oxygen and those showing no diving reaction characteristics when the water was disturbed were considered dead. For IGR insecticides Dudim and Baycidal, daily inspections were carried out until adult emergence, dead larvae were recorded. The distortion was calculated. Results were excluded from analysis if mortality rate was above 20%. In addition, if the percentage ranged between 5% and 20%, the mortality was corrected using the Abbott's formula [18]. Data from larval bioassays were subjected to probit analysis [19]. The concentrations of agents that killed 50% and 90% of mosquito larvae in 24 h ($LC_{50}$ and $LC_{90}$, respectively) were used to judge the larvicidal activity of the tested products [20]. The resistance status was determined according to World Health Organization [21].